

CLAIMS

What is claimed is:

1. A method for analyzing a sample to determine information about one or more molecules that may be present in said sample, said method comprising the steps of:

providing a sample in a detection zone wherein said sample comprises at least one molecule that is labeled with at least one fluorophore to provide a labeled molecule wherein said fluorophore remains within said detection zone for a detection period, said fluorophore being capable of emitting a signal wherein said signal has properties comprising wavelength, intensity, lifetime, and polarization;

exposing said labeled molecule to radiation within said detection zone wherein said radiation has properties comprising wavelength, intensity, lifetime and polarization;

modulating at least one of the properties of said radiation by changing said at least one property from an initial state to at least one subsequent state and then changing said at least one property back from said at least one subsequent state to said initial state wherein said modulating of said one or more properties occurs over a modulation period and wherein said modulation period is equal to or less than said detection period;

measuring at least one of said properties of said signal emitted by said fluorophore while said fluorophore is located within said detection zone to obtain an emission profile; and

using said emission profile to determine information about said one or more molecules that may be present in said sample.

2. A method for analyzing a sample according to claim 1 wherein the size of said detection zone is from 50 nm to 20 μ m.

3. A method for analyzing a sample according to claim 1 wherein said modulation period is at least ten times smaller than said detection period.

4. A method for analyzing a sample according to claim 1 wherein said modulation period is less than 1 millisecond

5. A method for analyzing a sample according to claim 1 wherein said sample comprises at least one molecule that is not labeled with a fluorophore and wherein said emission profile is used to determine information about said molecule that is not labeled with a fluorophore.

6. A method for analyzing a sample according to claim 1 wherein said labeled molecule comprises at least two different fluorophores.

7. A method for analyzing a sample according to claim 1 wherein said sample comprises at least two different labeled molecules.

8. A method for analyzing a sample according to claim 7 wherein said sample comprises at least three different labeled molecules.

9. A method for analyzing a sample according to claim 8 wherein said sample comprises at least four different labeled molecules.

10. A method for analyzing a sample according to claim 6 wherein said labeled molecule comprises at least three different fluorophores.

11. A method for analyzing a sample according to claim 10 wherein said labeled molecule comprises at least four different fluorophores.

12. A method for analyzing a sample according to claim 1 wherein said at least one labeled molecule comprises one or more molecule selected from the group consisting of nucleic acids, proteins, nucleotides, peptides, fluorophore-chelator conjugates, peptide nucleic acids, lipids, sugars, and hybrids thereof.

13. A method for analyzing a sample according to claim 5 wherein said at least one molecule that is not labeled comprises one or more molecules selected from

the group consisting of nucleic acids, proteins, nucleotides, peptides, peptide nucleic acids, lipids, sugars, and hybrids thereof..

14. A method for analyzing a sample according to claim 6 wherein said labeled molecule comprises a molecule selected from the group consisting of nucleic acids, proteins, nucleotides, peptides, fluorophore-chelator conjugates, peptide nucleic acids, lipids, sugars, and hybrids thereof..

15. A method for analyzing a sample according to claim 1 wherein said step of using said emission profile to determine information about said one or more molecules that may be present in said sample comprises calculating ratiometric expressions from said signals emitted by the said fluorophore and forming one or more histograms.

16. A method for analyzing a sample according to claim 1 wherein the number of molecules present in said detection zone at any one time is less than 10.

17. A method for analyzing a sample according to claim 5 wherein said emission profile is used to determine information comprising information about any interaction between said labeled molecule and said molecule that is not labeled.

18. A method for analyzing a sample according to claim 6 wherein said emission profile is used to determine information about the structure of said labeled molecule.

19. A method for analyzing a sample according to claim 1 wherein the property of said radiation that is modulated comprises said wavelength.

20. A method for analyzing a sample according to claim 1 wherein said modulating step comprises changing said at least one property from said initial state to at least two subsequent states before changing said at least one property back to said initial state.

21. A method for analyzing a sample to determine information about one or more molecules that may be present in said sample, said method comprising the steps of:

providing a sample in a detection zone wherein said sample comprises at least one molecule that is labeled with at least one fluorophore to provide a labeled molecule, said fluorophore being capable of emitting a signal wherein said signal has properties comprising wavelength, intensity, lifetime and polarization and wherein said one or more molecules undergo a change in state between a first molecular state and a second molecular state during a period of time while said molecule(s) are in said detection zone;

exposing said labeled molecule to radiation within said detection zone wherein said radiation has properties comprising wavelength, intensity, lifetime and polarization;

modulating at least one of the properties of said radiation by changing said at least one property from an initial state to at least one subsequent state and then changing said at least one property back from said at least one subsequent state to said initial state wherein said modulating of said one or more properties occurs over a modulation period and wherein said modulation period is equal to or less than the period of time during which said molecule changes from said first molecular state to said second molecular state;

measuring at least one of said properties of said signal emitted by said fluorophore while said fluorophore is located within said detection zone to obtain an emission profile; and

using said emission profile to determine information about said change in state of said one or more molecules that may be present in said sample.

22 A method for analyzing a sample according to claim 21 wherein said period of time during which said change in state takes place is at least ten times greater than said modulation period.

23. A method for analyzing a sample according to claim 22 wherein said modulation period is less than 250 microseconds.

24. A method for analyzing a sample according to claim 21 wherein said sample comprises at least one molecule that is not labeled with a fluorophore and wherein said emission profile is used to determine information about said molecule that is not labeled with a fluorophore.

25. A method for analyzing a sample according to claim 21 wherein said labeled molecule comprises at least two different fluorophores.

26. A method for analyzing a sample according to claim 21 wherein said sample comprises at least two different labeled molecules.

27. A method for analyzing a sample according to claim 26 wherein said sample comprises at least three different labeled molecules.

28. A method for analyzing a sample according to claim 27 wherein said sample comprises at least four different labeled molecules.

29. A method for analyzing a sample according to claim 25 wherein said labeled molecule comprises at least three different fluorophores.

30. A method for analyzing a sample according to claim 29 wherein said labeled molecule comprises at least four different fluorophores.

31. A method for analyzing a sample according to claim 21 wherein said at least one labeled molecule comprises one or more molecule selected from the group consisting of nucleic acids, proteins, nucleotides, peptides, fluorophore-chelator conjugates, peptide nucleic acids, lipids, sugars, and hybrids thereof..

32. A method for analyzing a sample according to claim 24 wherein said at least one molecule that is not labeled comprises one or more molecules selected from the group consisting of nucleic acids, proteins, nucleotides, peptides, peptide nucleic acids, lipids, sugars, and hybrids thereof..

33. A method for analyzing a sample according to claim 25 wherein said labeled molecule comprises a molecule selected from the group consisting of nucleic acids, proteins, nucleotides, peptides, fluorophore-chelator conjugates, peptide nucleic acids, lipids, sugars, and hybrids thereof..

34. A method for analyzing a sample according to claim 21 wherein said step of using said emission profile to determine information about said change in state of said one or more molecules that may be present in said sample comprises calculating ratiometric expressions from said signals emitted by the said fluorophore and forming one or more histograms.

35. A method for analyzing a sample according to claim 21 wherein the number of individual molecules present in said detection zone at any one time is greater than ten.

36. A method for analyzing a sample according to claim 24 wherein said emission profile is used to determine information comprising information about any interaction between said labeled molecule and said molecule that is not labeled.

37. A method for analyzing a sample according to claim 25 wherein said emission profile is used to determine information about the structure of said labeled molecule.

38. A method for analyzing a sample according to claim 21 wherein the property of said radiation that is modulated comprises said wavelength.